In Search of AIDS-Resistance Genes

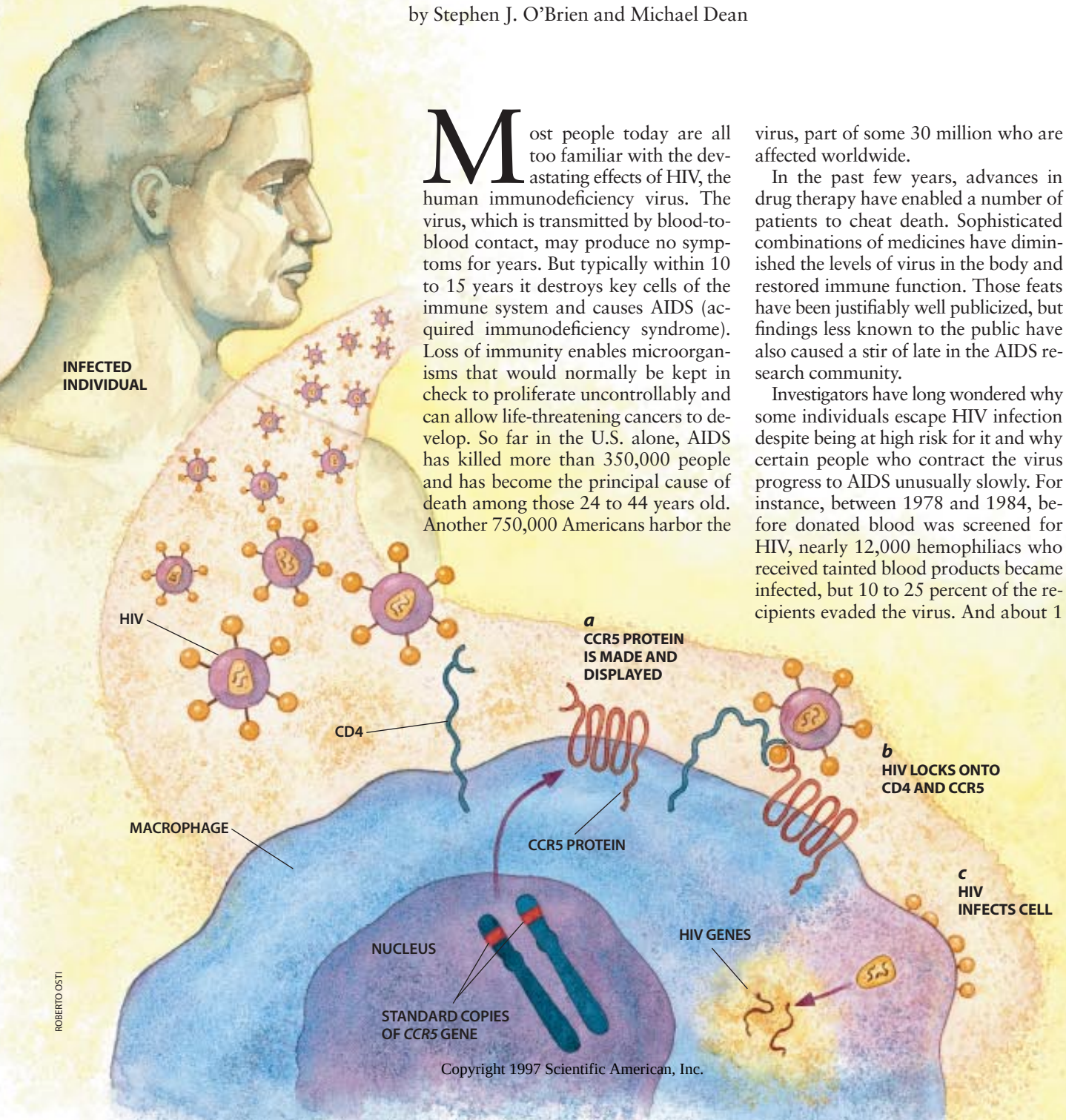
A genetic trait that protects against AIDS has now been uncovered, and others are emerging. The findings open entirely new avenues for developing preventives and therapies

by Stephen J. O'Brien and Michael Dean

Most people today are all too familiar with the devastating effects of HIV, the human immunodeficiency virus. The virus, which is transmitted by blood-to-blood contact, may produce no symptoms for years. But typically within 10 to 15 years it destroys key cells of the immune system and causes AIDS (acquired immunodeficiency syndrome). Loss of immunity enables microorganisms that would normally be kept in check to proliferate uncontrollably and can allow life-threatening cancers to develop. So far in the U.S. alone, AIDS has killed more than 350,000 people and has become the principal cause of death among those 24 to 44 years old. Another 750,000 Americans harbor the virus, part of some 30 million who are affected worldwide.

In the past few years, advances in drug therapy have enabled a number of patients to cheat death. Sophisticated combinations of medicines have diminished the levels of virus in the body and restored immune function. Those feats have been justifiably well publicized, but findings less known to the public have also caused a stir of late in the AIDS research community.

Investigators have long wondered why some individuals escape HIV infection despite being at high risk for it and why certain people who contract the virus progress to AIDS unusually slowly. For instance, between 1978 and 1984, before donated blood was screened for HIV, nearly 12,000 hemophiliacs who received tainted blood products became infected, but 10 to 25 percent of the recipients evaded the virus. And about 1



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percent of individuals who carry HIV remain relatively healthy, with few or no symptoms and with adequate immune functioning, for atypically long spans of 15 years or more.

The recent findings reveal that some people who are partly or fully resistant to HIV infection owe their good fortune to their genes—or, more precisely, to possession of a particular variant of a gene involved in immunologic function. This discovery has already sparked intensive efforts to translate the new genetic understanding into innovative strategies for preventing and controlling HIV infection. (We should note that we are using the term "HIV" to mean HIV-1, the virus responsible for most AIDS worldwide. Another form, HIV-2, causes AIDS more slowly and is restricted to certain parts of Africa; genetic resistance to HIV-2 has not yet been studied.)

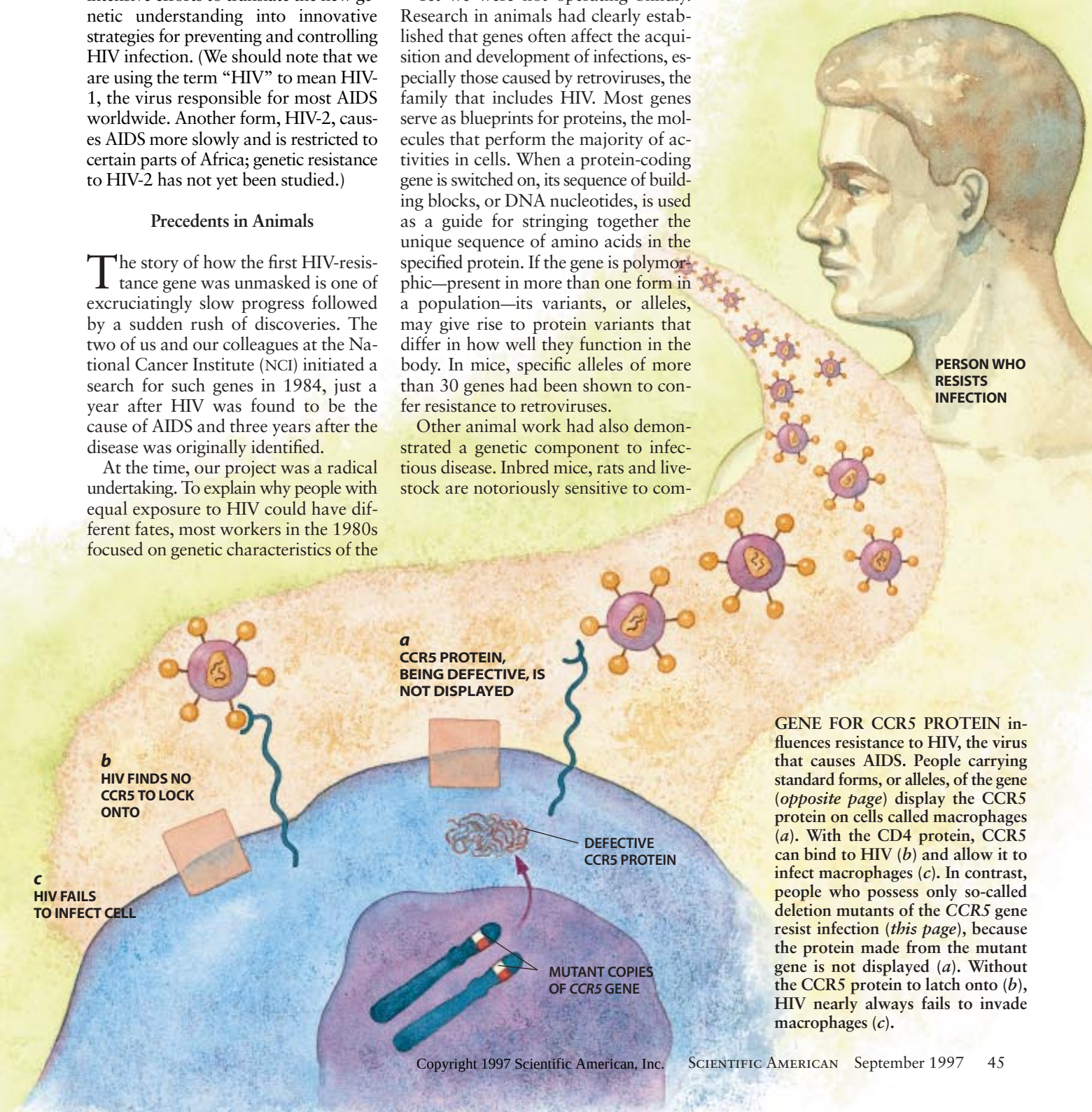
Precedents in Animals

The story of how the first HIV-resistance gene was unmasked is one of excruciatingly slow progress followed by a sudden rush of discoveries. The two of us and our colleagues at the National Cancer Institute (NCI) initiated a search for such genes in 1984, just a year after HIV was found to be the cause of AIDS and three years after the disease was originally identified.

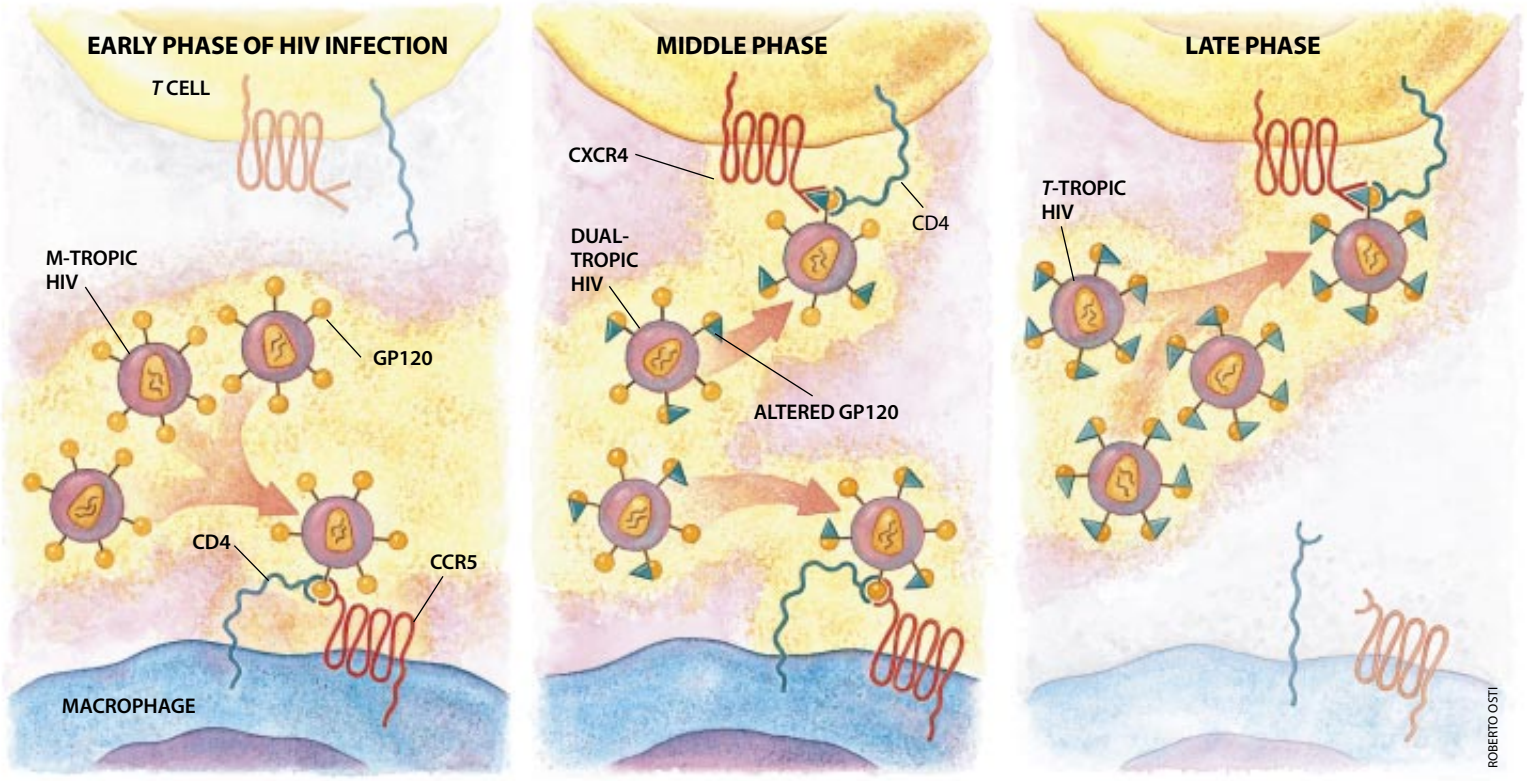
At the time, our project was a radical undertaking. To explain why people with equal exposure to HIV could have different fates, most workers in the 1980s focused on genetic characteristics of the virus (such as variations in the virulence of different strains) or on nongenetic "co-factors" that might influence the disease-causing power of the virus (such as infection of the host by another microbe). And we had little solid evidence that humans could possess genetic protection from AIDS. Indeed, certain of our colleagues doubted we would find anything on our genetic "fishing" expedition, a hunt on which we were wagering considerable time and resources.

Yet we were not operating blindly. Research in animals had clearly established that genes often affect the acquisition and development of infections, especially those caused by retroviruses, the family that includes HIV. Most genes serve as blueprints for proteins, the molecules that perform the majority of activities in cells. When a protein-coding gene is switched on, its sequence of building blocks, or DNA nucleotides, is used as a guide for stringing together the unique sequence of amino acids in the specified protein. If the gene is polymorphic—present in more than one form in a population—its variants, or alleles, may give rise to protein variants that differ in how well they function in the body. In mice, specific alleles of more than 30 genes had been shown to confer resistance to retroviruses.

Other animal work had also demonstrated a genetic component to infectious disease. Inbred mice, rats and livestock are notoriously sensitive to communicable disorders, mainly because inbreeding leaves them with a limited repertoire of disease-resistance alleles. In outbred groups, some fraction of a population is likely to have an allele that protects against a given pathogen; that allele will enable its owners to survive an epidemic and perpetuate the group. Because human populations are genetically diverse, we suspected that they, like other outbred species, possessed many powerful disease-resistance alleles.



GENE FOR CCR5 PROTEIN influences resistance to HIV, the virus that causes AIDS. People carrying standard forms, or alleles, of the gene (*opposite page*) display the CCR5 protein on cells called macrophages (*a*). With the CD4 protein, CCR5 can bind to HIV (*b*) and allow it to infect macrophages (*c*). In contrast, people who possess only so-called deletion mutants of the *CCR5* gene resist infection (*this page*), because the protein made from the mutant gene is not displayed (*a*). Without the CCR5 protein to latch onto (*b*), HIV nearly always fails to invade macrophages (*c*).



HIV'S AFFINITY FOR IMMUNE CELLS changes over time inside infected patients. Initially the virus is "M-tropic" (*left*): it favors macrophages, which it invades by binding (through its gp120 protein) to the molecules CD4 and CCR5 on the macrophage surface. Eventually, however, HIV can become "dual-tropic" (*center*). Such strains produce gp120 molecules able to recognize the CXCR4 protein on CD4-bearing *T* cells and may infect both macrophages and *T* cells. Still later, the bulk of the viral population can switch its preference to the CXCR4 receptor and become "*T*-tropic" (*right*). *T*-tropic viruses readily destroy infected *T* cells and thereby contribute to the collapse of the immune system and the onset of AIDS.

Those alleles, perhaps including defenses against HIV, simply remained to be discovered.

Further, although few pathogen-resisting alleles had been defined convincingly in humans, several epidemiological studies had noted a strong genetic influence on disease susceptibility. For instance, one analysis showed that if a biological parent of an adoptee died of an infectious disease before age 50, the adoptee had a markedly increased risk of also dying from an infection.

Unfortunately, science had provided no simple blueprint for finding HIV-resistance alleles in humans. We therefore combined knowledge and techniques from three disparate disciplines: AIDS epidemiology, human molecular genetics and population genetics theory.

High-Tech Gene Prospecting

First, we needed a source of genes from the populations of interest to us, such as individuals at high risk for HIV infection who did or did not become infected after exposure to the virus. If the two groups differed in their genetic makeup—in their alleles for specific genes—we would suspect that the genes displaying the variation influenced susceptibility to HIV infection.

To obtain human DNA for study, we joined forces with public health epidemiologists who were trying to track the pattern of the still new epidemic. As part of that effort, the epidemiologists were enlisting cohorts, or groups of several hundred individuals, at high risk for HIV infection—notably, homosexual men, users of intravenous drugs and hemophiliacs who had received contaminated blood products. These cohorts were to be monitored for years by physicians, who (with the patients' permission) would supply blood, tissue samples and case reports to researchers. As blood was collected, our cell biology team, led by Cheryl Winkler, carefully produced immortal lines of cultured cells that would provide an unlimited supply of DNA for genetic testing.

To determine which genes to compare, we took advantage of recent advances in gene mapping, a set of procedures that pinpoints the location of genes on chromosomes and determines their nucleotide sequences. More than 6,000 of the approximately 50,000 to 100,000 genes in human chromosomes have now been mapped. Back in 1984 fewer than 1,000 had been found. Nevertheless, to test even 1,000 genes in our AIDS cohorts was an impossible task.

We narrowed the choice by drawing on established knowledge of how retroviruses behave in their hosts. The host is always an unsuspecting collaborator in establishing infection and enabling pathogens to spread through tissues. To enter cells, all viruses must recognize (bind to) certain proteins encoded by host genes and displayed on the cell. These proteins normally act as receptors for other host molecules, but viruses can co-opt the receptors, using them as springboards for entry into a cell.

Once in a cell, retroviruses insidiously insert their genes into a host's chromosomes. They thereby ensure that viral genes—which can direct the synthesis of an endless supply of viral particles—are passed to each new generation of cells whenever the initial host cell replicates. Here, again, the viruses require help from the host. They must recruit several cellular enzymes to splice viral genes into chromosomes, to produce fresh viral particles and even to evade the host's immune defenses.

With such understanding to guide us, we originally decided to concentrate on

about 50 genes whose proteins could potentially influence HIV's life cycle. We also examined 250 polymorphic (variable) DNA segments that had been identified in chromosomal sites between genes. If our subjects differed in these segments, those differences would indicate that alleles of nearby genes might also vary systematically between the groups. We could then perform a fairly narrow search for those genes and try to determine their function in cells and their role in HIV infection.

Finally, to pinpoint genetic traits that confer resistance to HIV, we borrowed strategies from human population genetics. We divided each cohort into two groups, according to selected aspects of their health—for example, those infected with HIV versus those who remained free of it after extensive exposure; infected patients who progressed to AIDS rapidly versus those who progressed slowly if at all; or infected patients who acquired a specific AIDS-related disease (such as *Pneumocystis carinii* pneumonia or Kaposi's sarcoma) versus those who did not.

Having made these divisions, we compared how often each known allele or polymorphic segment appeared in the groups. We also compared what are called genotypes. An individual inherits two copies of all genes outside the sex chromosomes (one copy from the mother and one from the father). The pair of alleles at a particular chromosomal locus, or gene address, constitutes the genotype. Someone who inherits two identical alleles of a given gene is said to be a homozygote; someone who inherits two distinct alleles is said to be a heterozygote. In our screening tests, we noted the percentage of patients in each group who were homozygous for a known allele and the percentage of patients that were heterozygous. Appreciable differences in allele or genotype frequencies, or both, in two subject groups would indicate that the gene under study probably accounted for the divergent fates of the subject groups.

For years we continued to add more patients, more genes, more polymorphic segments and more sophisticated computer programs to analyze the data. Periodically, we thought we noted genetic differences, but they nearly always evaporated under close inspection. Meanwhile we monitored the many advances in understanding of human immunology and in the behavior of HIV in the body, always seeking ideas for other genes to study. Late in 1995 and early in 1996—more than a decade after we began this massive and tedious effort—cracks finally appeared in the dike.

Good Clues, at Last

Those cracks were created by other research teams who resolved two long-standing mysteries relating to HIV's molecular interaction with host cells. With those solutions came clues to genes involved in resistance to HIV.

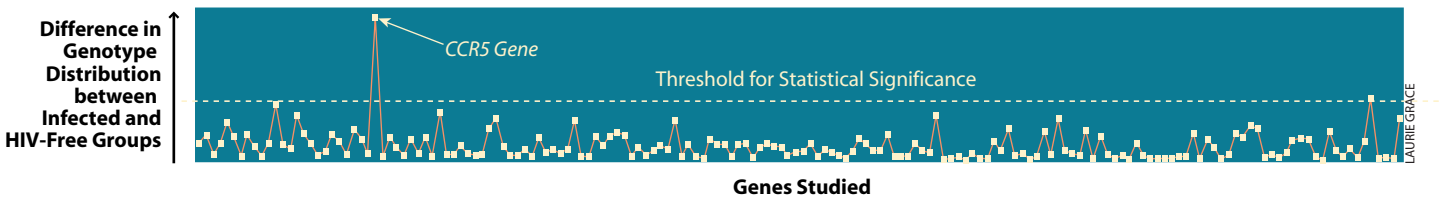
By the mid-1990s scientists and nonscientists alike were well aware that HIV caused immune deficiency mainly by depleting white blood cells known as *T* lymphocytes that displayed a protein called CD4 on their surface. These *T* cells normally orchestrate many aspects of the immune response to viruses. It was also known, albeit less widely, that HIV can infect and persist for years in another class of CD4-carrying immune cells called macrophages. HIV does not destroy macrophages and finds a safe haven in them.

The CD4 molecules on *T* lymphocytes and on macrophages usually participate in signaling between immune cells. But when HIV enters the picture, CD4 molecules bind to a sugary protein (gp120) protruding from HIV's outer envelope and, in so doing, help the virus to gain entry into the bound cells. Yet experiments had shown that CD4, though necessary for HIV infiltration of cells, was not sufficient; the cells also had to display at least one more protein to which the virus could bind. More than 10 years after the discovery of HIV, however, scientists still had no clue to the nature of that second receptor.

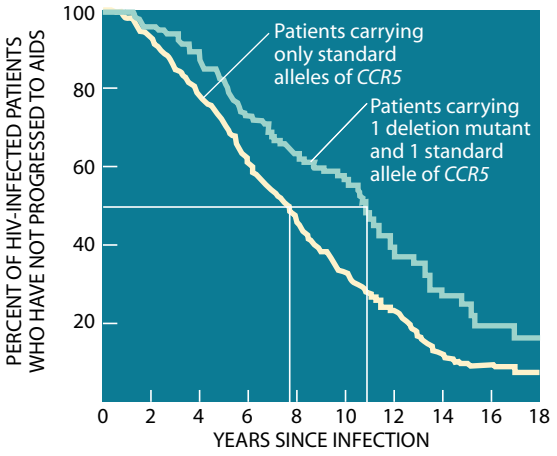
The other puzzle related to a discovery reported in 1986 by Jay A. Levy of the University of California at San Francisco. He found that a class of *T* lymphocytes displaying a different protein—CD8—secreted molecules, termed suppressive factors, that blocked HIV from invading normally susceptible cells in culture. Suppressive factors that limited virus infection had also been shown to exist in African monkeys that harbored SIV (the simian form of HIV) yet did not advance to AIDS, as well as in people who survive HIV infection for an unusually long time. The identity of these sundry suppressive factors remained to be determined, however.

In December 1995 Robert C. Gallo, then at the NCI, and other collaborators announced that they had identified three related suppressive factors that could block infection by HIV variants that prefer to colonize macrophages (so-called M-tropic strains). All three factors turned out to be known chemokines: short strings of amino acids responsible for luring immune cells to injured or diseased tissues.

	2 Copies of Standard *CCR5* Allele	2 Copies of Mutant *CCR5* Allele	1 Standard and 1 Mutant *CCR5* Allele
HIV-Infected Individuals	85 percent	0 percent	15 percent
Uninfected Individuals	83 percent	3 percent	14 percent



DIFFERENCES IN THE GENETIC PROFILE of two populations (*graph*) implicated the *CCR5* gene in resistance to HIV infection. The authors first identified the genotypes, or combinations of alleles, for each of 170 genes in a group of HIV-infected patients and, separately, in a group of uninfected individuals. For instance, they measured the percentages of people having two copies of the normal *CCR5* allele, two copies of the truncated, deletion mutant, or one copy of each (*table*). Then, for every gene, they plotted the statistical difference (*dots*) in the genotype distribution between the two populations under study. Of all the genes that were examined, only the difference for the *CCR5* gene turned out to be highly significant.



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COMPARISON of how long HIV-infected individuals lived without progressing to AIDS revealed that patients harboring one deletion mutant of the *CCR5* gene (*green line*) avoided AIDS longer than patients carrying only standard *CCR5* alleles (*yellow line*). For instance, it took about 11 years for 50 percent of the first group to advance to AIDS but about eight years for half of the second group to reach that point.

Many investigators still grappling with the first puzzle—the search for HIV's second receptor—understood that chemokines work their effects on defensive cells by binding to surface proteins. It seemed possible that the chemokines isolated by Gallo's group—named RANTES, MIP-1α and MIP-1β—might interfere with HIV entry into immune cells by binding to and blocking some cell surface protein that HIV required for access to the interior. In other words, the cell-surface receptor (or receptors) for Gallo's chemokines could well lead a double life as the second receptor for HIV on macrophages and perhaps on other CD4-bearing cells.

The notion defied immediate testing because the cellular receptor for RANTES and its cousins had not yet been isolated. But discoveries reported early in 1996 made such tests possible and provided us, and others, with new genes to screen as resistance factors.

First, Edward A. Berger and his colleagues at the National Institute of Allergy and Infectious Diseases isolated the second receptor for HIV variants that prefer to colonize *T* lymphocytes (*T*-tropic strains). It was a chemokine receptor, albeit one (now called CXCR4) that bound a chemokine distinct from RANTES, MIP-1α and MIP-1β. If Gallo's findings had not convinced AIDS researchers that chemokine receptors played a part in HIV infectivity, Berger's results drove the point home.

Almost simultaneously, Michael Samson and Marc Parmentier of the Free University of Brussels and their collaborators isolated the gene for a receptor onto which RANTES, MIP-1α and MIP-1β all hook when they draw defensive cells to damaged tissue. Within two months, five separate groups proved that the encoded protein, now known as CCR5, was also the elusive second receptor for M-tropic strains of HIV.

Combined with observations from other studies, the new chemokine receptor findings critically refined understanding of how HIV infections become established and progress. HIV initiates infection by setting up residence primarily in macrophages. It enters these cells by linking its gp120 protein with two receptors on macrophages: CD4 and CCR5. Once inside the macrophages, HIV synthesizes large quantities of virus and challenges the immune system to its limits.

Years later the constantly mutating virus can alter the gene for gp120 in a way that causes the gp120 protein to change its second-receptor allegiance. The genetic change causes the region that recognizes CCR5 to bind more effectively to CXCR4 on *T* lymphocytes. Now the HIV population becomes dominated by *T*-tropic variants—those preferring to infect *T* cells.

This shift in attraction soon becomes deadly, because *T*-tropic viruses kill the cells they infect. Not surprisingly, the shift is often followed swiftly by an overall drop in CD4 *T* cell concentrations in patients and, simultaneously, by the onset of the opportunistic infections and cancers that for many years defined progression to AIDS. Today the Centers for Disease Control and Prevention formally defines AIDS by the presence of AIDS-defining illnesses or by a drop in CD4 *T* cells to fewer than 200 per cubic millimeter of blood; normal levels are about 1,000 per cubic millimeter.

The Expedition Succeeds

As soon as we knew that CCR5 and CXCR4 were co-receptors for HIV, we immediately decided to see whether the genes for those proteins affected resistance to HIV in our cohorts. To pursue this idea, we had to determine whether the *CCR5* and *CXCR4* genes were polymorphic. If everyone had identical versions of those genes, the genes could not account for differences in susceptibility to HIV.

All copies of the *CXCR4* gene we examined were the same. But in July 1996 Mary Carrington of our group discovered that a major variant of the normal *CCR5* gene occurred in about one in five individuals. Comparisons of the nucleotide sequences of the two *CCR5* alleles revealed that the less common one was missing 32 nucleotides. Because of the way the genetic code works, we knew that the loss would result in the premature creation of a "stop" code in the gene and would, in turn, cause the cells to manufacture a severely foreshortened version of the CCR5 protein.

When we divided nearly 2,000 high-risk patients into infected and noninfected groups and compared their *CCR5* genotypes, we found dramatic differences. Some 3 percent of the noninfected individuals carried only the deletion mutant of *CCR5* in their cells (that is, were homozygous for the mutant). In contrast, not one patient out of 1,343 in the infected group was homozygous for the deletion mutant. The difference—which indicated homozygosity for the deletion mutant was protective against HIV—was highly significant statistically and was certainly no coincidence.

Moreover, the apparent protection provided by having solely mutant *CCR5* alleles did not depend on the route of infection: no hemophiliacs, homosexuals or drug users who were homozygous contracted HIV. We suspected that homozygosity for the deletion mutant shielded patients because they manufactured only truncated CCR5 proteins that either failed to reach the cell surface or were so deformed that they could not dock with HIV.

Within a few weeks after submitting a paper on these remarkable findings to the journal *Science*, we learned we were not alone in searching for polymorphisms in chemokine receptors. Nathaniel R. Landau and Richard A. Koup of the Aaron Diamond AIDS Research Center in New York City and their co-workers had independently discovered the same 32-base-pair deletion allele. They had been studying a group of homosexual men who had many high-risk sexual exposures to HIV but had never become infected. Examination of white

The Mysterious Natural History of the Resistance Allele

The HIV-resistance allele, or deletion mutant, of the *CCR5* gene is not distributed equally among the world's peoples. It is virtually absent in African and eastern Asian populations and in Native Americans and is rare in African-Americans [*see second column in table below*]. It is, however, fairly prevalent among Caucasians (descendants of the early settlers of Europe and western Asia).

Yet even among Caucasians the distribution varies. A plot of the allele's frequency among Caucasians in Eurasia [*see map below*] reveals a gradient, or cline, that is highest in the north and drops to an undetectable level in Saudi Arabia. The frequency is calculated by counting the number of mutant copies in a population and dividing by the total of all *CCR5* copies—the sum of mutant and standard copies combined.

These patterns answer some questions and raise others about the origin and prevalence of the mutant, which codes for a defective CCR5 protein. The apparent absence of the mutant in Africa indicates that it arose some time after humans left Africa—a split widely believed to have taken place 130,000 to 200,000 years ago. But what caused the deletion mutant to reach such a high frequency in Caucasians, and when did that event occur?

The surprisingly high frequency of the allele in parts of Europe and Asia suggests that some devastating event in these locales gave originally rare individuals who harbored the mutant a dramatic survival advantage. Those individuals then lived to reproduce, causing the fraction of the population bearing the allele to become larger than before. As survivors of this historic cataclysm procreated, their *CCR5* mutation persisted and accumulated to higher levels.

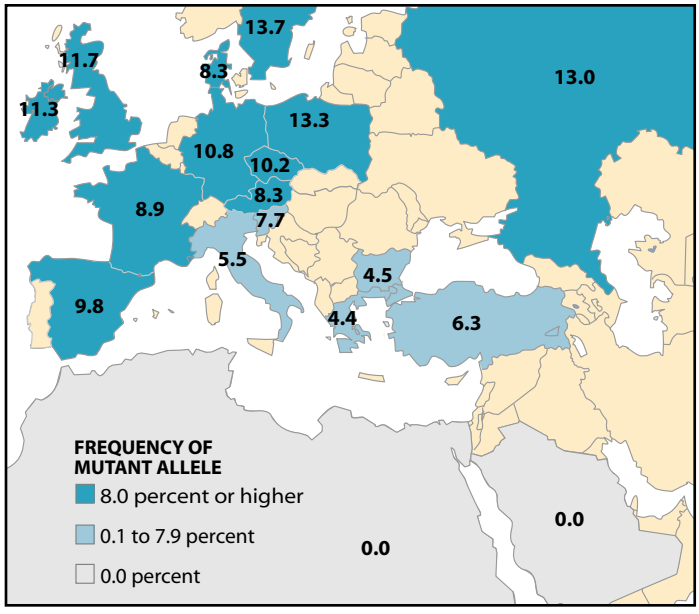
We suspect that the catastrophic event was a major epidemic caused by an agent that, like HIV, makes use of the normal CCR5 protein, but not the defective form, to infect cells. This hypothesis makes sense to us because rare alleles often become more common in animals after they provide resistance against a newly encountered pathogen. And the longer the epidemic lasts, the higher the allele frequency rises.

We also think the Eurasian epidemic occurred very long ago. Indeed, using genetic dating methods, we have estimated that the catastrophic challenge struck about 4,300 years ago and certainly no more recently than 1,200 years ago. The mutant is less common in southern Eurasia than in the north, perhaps because the eye of the epidemic was concentrated in the north.

The European and western Asian heritage of many Americans would explain why a relatively high fraction of American Caucasians harbor the allele: their ancestors brought it with them as part of their genetic endowment. And some African-Americans possess the allele, even though most Africans do not, probably because of recent intermarriage between Africans and Caucasians in the Americas.

We can only wonder, however, at that pathogen's identity. An agent like HIV, which kills more than 90 percent of its victims, could have been responsible. Today's HIV was not the culprit—it exploded in human populations within the past 20 years—although an ancient, undocumented HIV outbreak might have occurred. Other possibilities include organisms responsible for cholera, tuberculosis or the flu. The microbe that caused the bubonic plague of the 14th century, once considered a reasonable contender, probably was not at fault, though. It did not attack Europe until about 600 years ago. *—S.J.O'B. and M.D.*

Population	Frequency of Mutant Allele (percent)	Frequency of Genotypes (percent)		
	Mutant Allele	2 Copies of Standard Allele	2 Copies of Mutant Allele	1 Standard and 1 Mutant Allele
Caucasian-European	10.0	81.0	1.0	18.0
Caucasian-American	11.1	79.0	1.2	19.7
African-American	1.7	96.6	0.0	3.3
Native American; African; East Asian	0.0	100.0	0.0	0.0



blood cells from two of these men indicated that the CCR5 protein was absent from the cell surface. A look at the nucleotide sequence of the *CCR5* genes revealed that both men were homozygous for the deletion mutant. Further, in other work, Samson and Parmentier's team had searched for and failed to turn up any homozygotes for the deletion allele in a group of 743 HIV-infected people. (Those two reports appeared in August 1996, ours in September.)

Subsequent studies uncovered no homozygotes among Africans, Asians or African-Americans but indicated that 1 to 2 percent of Caucasian-Americans—those descended from Europeans or western Asians—are homozygous for the mutation. Further, when we looked at the genotypes of uninfected people known to have had extremely high exposure to HIV (through engaging in unsafe sex repeatedly or receiving high doses of HIV-contaminated clotting factors during treatment for hemophilia), we saw that as many as 20 percent of these individuals were homozygous for the deletion mutant. Resistance to infection in the other 80 percent must have come from other genetic or nongenetic sources.

It stood to reason that if two mutant *CCR5* genes provided complete protection from HIV, possession of one mu-

tant and one normal allele might provide partial protection, by halving the number of functional CCR5 proteins made by a cell. When we analyzed the time between infection and the appearance of AIDS-defining diseases, we found that the onset of overt AIDS was postponed for two to three years in HIV-infected individuals who carried one deletion allele. This delay was apparent both in homosexual men and in hemophiliacs. This heterozygous genotype (which occurs in approximately 20 percent of Caucasian-Americans) also delayed the time at which CD4 *T* cell levels fell below 200 per cubic millimeter of blood.

The excitement was overwhelming. The deletion mutant, when inherited from both parents, did indeed appear to provide powerful genetic protection against HIV even after repeated exposures. And inheritance of a single deletion mutant could slow progression to AIDS in infected individuals. These results implied that treatments able to block the interaction of HIV with the normal CCR5 protein might help protect healthy people from HIV infection or delay the advance to AIDS in people who have already contracted the virus.

For years, pharmaceutical companies had focused their anti-HIV therapeutic efforts on the virus alone, giving little attention to how the host's cellular machinery collaborates in establishing chronic disease. The drugs used in combination therapy, for example, interfere directly with the activities of HIV itself, such as by preventing certain of its enzymes from functioning. The new genetic results suggested that targeting the host's complicity in the progression to AIDS could open previously unimagined avenues for controlling HIV replication in infected patients or for preventing HIV infection in the first place.

Implications for Treatment

Not surprisingly, many investigators quickly began considering ways to keep HIV and the CCR5 protein from interacting. In theory, such strategies could involve substances that sheathe gp120. In practice, however, most efforts are searching for ways to plug the HIV binding site on CCR5.

An initial concern was that blocking CCR5 would be dangerous—that it might impair immunity by making macrophages deaf to the call of RANTES and related chemokines. But that worry was soon allayed. Individuals who possess two mutant alleles have no obvious immune dysfunction or tissue pathology and appear to be quite healthy. Evidently, other chemokine receptors can compensate for the lack of CCR5. Two of them (CCR2B and CCR3) can also serve as co-receptors for HIV, although they generally do not perform that nefarious job nearly as effectively as CCR5.

Among the therapeutic strategies under consideration is direct delivery of molecules that would obstruct CCR5's binding site for HIV. Such molecules could include chemokines or synthetic derivatives of chemokines. For instance, an international team of investigators has developed a modified chemical derivative of RANTES that shows promise in test-tube studies. Other molecular "plugs" could include synthetic antibodies—larger immune molecules that would specifically home to CCR5 and bar attachment by HIV.

Additional approaches involve vaccinating people with fragments of CCR5 that could induce the recipient's immune system to produce its own CCR5-binding antibodies. Alternatively, researchers could use genetic engineering to provide macrophages with new genes whose products would prevent CCR5 from being made or would stop CCR5 from serving as a docking site for HIV.

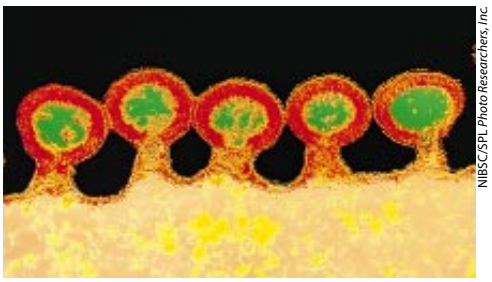
For some patients facing imminent death—such as those in the final stage of AIDS who also have lymphoma—our group is considering modifying a radical treatment increasingly applied to advanced cases of blood or breast cancers. When curing these cancers is the aim, patients are given extremely high doses of chemotherapy or radiation to eradicate all cancer cells. Because that therapy destroys the blood-producing cells of the bone marrow (including the ones that give rise to the immune system), physicians then reconstitute the patient's immune system by delivering healthy, tissue-compatible marrow.

In the case of AIDS patients, we would aim to destroy all HIV-infected blood cells and then to rescue the patient with bone marrow from donors who are homozygous for the deletion mutant of the *CCR5* gene. This last step would, we hope, help protect the patient from new HIV infection and also help prevent the cell-to-cell spread of any HIV particles that somehow survived the HIV-destroying therapy.

The idea of simultaneously curing pa-

Other Influences on HIV Progression

As we have shown, genes can certainly influence whether someone who is exposed to HIV becomes infected and progresses rapidly to AIDS. But other factors—including properties of the host and of the virus—can play a role as well.



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HIV PARTICLES bud from an infected cell. Some strains are more aggressive than others.

Some people mount a stronger immune attack on HIV right from the start, perhaps because they have previously been exposed to a virus that structurally resembles HIV. A powerful early response that sharply limits HIV levels in the host could conceivably eliminate the virus in some cases. The strength of the immune response can also influence the rate at which people who do become infected advance to AIDS.

Whether an individual is contending with other viruses may also affect susceptibility to HIV and the speed of AIDS onset. The presence of concomitant infections in the body leads to the production of substances called cytokines, some of which are thought to promote HIV entry into cells and HIV replication.

Finally, the viral strain itself can make a big difference in the rate at which HIV infection advances. Strains that are quite choosy about the cell types they will infect, that replicate and mutate relatively slowly and that do not kill host cells are likely to be least destructive to the immune system—at least at first. Even initially mild strains, however, may later mutate into more aggressive forms. *—S.J.O'B. and M.D.*

tients and giving them protection from residual or sequestered HIV holds great appeal, but we are approaching bone marrow therapy cautiously because of a few important concerns. For one thing, bone marrow transplants are inherently risky: immunologic differences between the donor and the recipient can cause rejection of the transplant or, worse, can cause the immune cells in the donor marrow to attack the tissues of the host and kill the patient.

In addition, in recent months, a few individuals have surfaced who are homozygous for the deletion mutant but who have nonetheless become infected with HIV. We do not yet know how the infection became established, but some signs indicate that these rare patients met with an unusual "hot," or highly virulent, *T*-tropic strain of the type that typically emerges only in the late stages of HIV infection.

Until now, conventional wisdom held that *T*-tropic viruses were unable to spread infection from one person to another. They seemed to be recognized and destroyed by the healthy immune system of newly exposed individuals. Successful infection was thought to require M-tropic viruses, which quietly multiplied to high levels in macrophages without eliciting destruction of those cells. Some evidence suggests that the patients who became infected even though they were homozygous for the deletion mutant were merely unlucky and simply encountered odd *T*-tropic strains that were able to circumvent immune defenses and establish infection without needing M-tropic strains to lay the groundwork. It is also possible, however, that the patients' innate resistance to M-tropic strains somehow sped up the transition of M-tropic strains to hot *T*-tropic types able to establish infection on their own.

If CCR5-mediated resistance to M-tropic strains actually encouraged HIV to turn hot, the finding would mean that bone marrow transplants—and, in fact, any preventives or therapies aimed at blocking HIV's access to CCR5—could backfire and encourage, instead of forestall, infection and advancement to AIDS. The fact that most people who are homozygous for the deletion allele avoid HIV infection instead of succumbing to severe *T*-tropic viruses is reassuring. Nevertheless, before physicians can routinely treat patients with antagonists of CCR5, investigators need to show that such drugs improve, rather than diminish, the likelihood of survival.

As scientists explore safe, effective ways to capitalize on the recent genetic findings, they also continue to look for other genetic factors that could suggest additional ways to shield people from AIDS. Indeed, our group has recently identified a variant of the *CCR2B* gene that even in a single copy delays the onset of AIDS by two or three years, just as heterozygosity for *CCR5* does. And earlier this year Jianglin He of the Dana-Farber Cancer Institute and his colleagues reported that the CCR3 protein promotes HIV entry into microglia (immune cells in the brain) and that blockade of the receptor prevents HIV infection of microglia in the laboratory.

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STEVE CROHN possesses only deletion mutants of the *CCR5* gene. Like most others with that same genetic profile who have had high exposure to HIV, he remains free of the virus. Investigators are working to develop treatments that will confer similar protection to people who carry standard copies of the *CCR5* gene.

After more than a decade of searching for genetic traits that provide protection from AIDS, we are indeed pleased by the quickening pace of discovery. But the main goal must be transforming genetic insights into novel ways to evade or attack HIV, a virus clever enough to destroy the very cells meant to eradicate it. Although therapeutic applications remain speculative for now, we are hopeful that the combined talents of researchers from many fields will provide a scientific recipe for reversing the deliberate progression of the AIDS epidemic. SA

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STEPHEN J. O'BRIEN and MICHAEL DEAN have collaborated for more than a decade. O'Brien has been chief of the Laboratory of Genomic Diversity at the National Cancer Institute since 1986. He is internationally recognized for his contributions in human and animal genetics, evolutionary biology, retrovirology and species conservation. With two other colleagues, O'Brien also founded and co-directs NOAHS, a Smithsonian Institution consortium of scientists and apprentices who apply biotechnology on behalf of species conservation. Dean is chief of the human genetics section of the Laboratory of Genomic Diversity, where he applies new genetic techniques to the study of complex human diseases. The authors dedicate this article to the memory of Daniel O'Brien, Stephen O'Brien's brother, who died from AIDS in 1994.

Further Reading

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Contrasting Genetic Influence of CCR2 and CCR5 Variants on HIV-1 Infection and Disease Progression. Michael W. Smith et al. in *Science* (in press).

